Surgical implantation of radio tags in three eel species (*Anguilla* spp.) in South Africa

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Running header: Surgical tag implantation in African freshwater eels

Abstract

Studies have reported poor survival of surgically-tagged freshwater fishes in warm African waters. This study aimed to assess the applicability of using radio telemetry (and surgical implantation of tags) for *Anguilla* spp. Nineteen yellow eels (*Anguilla bengalensis*, *A. marmorata* and *A. mossambica*) were surgically implanted with radio tags between October 2018 and January 2019 in the Thukela River, South Africa. Most eels were alive 6 months after tagging, and recaptured eels displayed advanced or complete healing at the incision site. Therefore, this method appears suitable for African freshwater eels.

KEYWORDS

tagging impacts, telemetry, tropical, fish behaviour, developing country

Four anguillid eel species occur in eastern Africa and the associated islands: *Anguilla bengalensis* (Gray 1831), *A. bicolor* McClelland 1844, *A. marmorata* (Quoy and Gaimard 1824) and *A. mossambica* (Peters 1852) (Skelton, 2001). In South Africa the frequency of occurrence of anguillid species increases northwards and reaches a peak in KwaZulu-Natal (KZN) Province, where all four species coexist in the same catchments (Hanzen *et al*., 2019). Knowledge on the behaviour of African eels in freshwater is sparse with no publications found to date. In Africa, eels are known to occupy a variety of habitats (Bell-Cross and Minshull, 1988), and their habitat use may vary with species and size, but little detailed evidence exists. Although there is concern over the population status of anguillids worldwide, a lack of ecological information makes conservation planning for African anguillids particularly challenging (Jacoby *et al*., 2015).

Telemetry (*sensu* Cooke *et al*., 2012; acoustic, VHF, UHF, GPS or passive transponders) is an effective method for gathering data on habitat use, movement and behaviour of fishes (Cooke *et al*., 2012). While its use has been fairly limited in African freshwaters, telemetry has been successfully used on several species of siluriform (Hocutt, 1989; Kadye and Booth, 2013), cichlid (Thorstad *et al*., 2004), cyprinid (Burnett *et al*., 2018), alestid (Baras *et al*., 2002; Økland *et al*., 2005) and protopterid (Mlewa *et al*., 2005) fishes. To date there have been no telemetry studies on freshwater eels (*Anguilla* spp.) in Africa.

Tag attachment is a crucial element of telemetry study design. Although invasive, surgical implantation into the body cavity is usually considered to be the best technique for long-term fish telemetry studies (Cooke *et al*., 2012). A low risk of mortality (Hirt-Chabbert and Young, 2012) and high retention rate (Zimmerman and Welsh, 2008) can be achieved, but this is variable across species and habitats, and trials of suitability are always recommended with new study species (Jepsen *et al*., 2002; Cooke *et al*., 2012). In Africa, surgical implantation of tags into freshwater fishes has been employed both successfully (Hocutt, 1989; Huchzermeyer *et al*., 2013; Howell *et al*., 2015) and less successfully, with high mortality rates (Økland *et al*., 2003) and tag loss (Økland *et al*., 2003; Mlewa *et al*., 2005) contributing to unsuccessful experiments. The high temperatures of African rivers are thought to contribute to a higher risk of infection that could later lead to mortality or tag loss (Økland *et al.,* 2003). Many radio-telemetry studies of African freshwater fishes have favoured the use of external radio tags, as handling time is reduced, which equates to lower associated stress levels, decreasing the risk of infection and tag rejection (e.g. Økland *et al.*, 2007; O'Brien *et al.,* 2013). However, due to the cryptic and refuge seeking behaviour of freshwater eels, internal telemetry tags result in higher retention rates (Cottrill *et al*., 2006). Based on this information, we aimed to internally tag three species of African freshwater eels to assess the applicability of this tagging technique for these species in a South African river.

The study was carried out in the Thukela catchment, which has the largest mean annual runoff in South Africa (DWAF, 2003), and is the largest catchment in KZN covering approximately 30,000 km^2 (DWAF, 2002). Although the catchment is regulated with several inter-basin water transfer schemes, the Thukela River itself is mostly free-flowing. The study was conducted in the middle reaches of the Thukela, on an approximately 6-km stretch of river in the Zingela Private Nature Reserve. Located approximatively 300 km from the sea, with no major obstacle downstream, our study area was expected to be within the distribution range of *A. marmorata*, *A. bengalensis* and *A. mossambica*. This stretch of river is characterised by a mixed bed alluvial channel and comprises a variety of habitats, including deep pools and fast, shallower habitats. The river is predominately turbid with visibility generally not exceeding 0.2 m (C. Hanzen, pers. obs.).

Very high frequency (VHF) radio telemetry was selected as it is most suitable to use in shallow rocky environments (maximum depth 5 m), a characteristic of the study area. While eels smaller than 550 mm and lighter than 220 g were available in our study stretch, the size for tagging was set at minimum of 550 mm or 475 g. Whereas the traditional ~ '2% of body mass rule' (Winter, 1983) would have allowed eels as small as 180 g to be tagged, it was judged insufficient in this study as the morphology of eel and the abdominal space was evaluated to be more of a limiting factor (Jepsen *et al*., 2004). All eels weighing less than 2075 g, except one of 4200 g, were tagged with F1580 tags with a whip antenna (24 × 13 × 7 mm, 3.6 g; Advanced Telemetry Systems, Isanti, USA) while for eels heavier than 2075 g, F1820 tags with a whip antenna were used (36 × 12 × 12 mm, 9.5 g). Expected transmission lives for these models were 284 (at 40 pulses per minute, ppm) days and 286 (40 ppm) days, respectively.

Animals ethics clearance for this study was obtained from the University of KwaZulu-Natal Animal Ethics Committee (AREC/012/017D). Eels for tagging were caught between October 2018 and January 2019 in the Thukela River at sites spread along the Zingela reach of river using commercial fyke nets (n = 12) set for 5 – 6 consecutive nights monthly. Nets were checked in the morning, suitable eels were selected and tagged immediately on the riverbank in the vicinity of the capture site under natural shade when available. Water temperature during the tagging procedure ranged from 22 °C to 27 °C. Individuals to be tagged were immersed in an aerated bucket filled with ~50 L of an anaesthetic solution in river water (2-phenoxyethanol, ~ 0.5 ml/l). Once anaesthetised, an eel was placed ventral side up in a PVC pipe which was longitudinally cut in half. As the eels were found to have a very quick recovery in fresh water, a continuous flow of anaesthetic water was applied over the gills for the duration of tagging. The tag was inserted into the abdominal cavity through a ~2 cm mid-ventral incision (Ovidio *et al*., 2013). To minimise the probability of eels biting at incision sutures and reduce the risk of damage to the liver (Økland and Thorstad, 2013), the incision was made at a position 25–30% of body length from the snout. The whip antenna was taken out through the abdominal wall with a hollow needle. The incision was closed with three simple interrupted sutures (CliniSolv 8224RC 2/0 24 mm 3/8 Circle Reverse cutting Monofilament Synthetic Absorbable Suture, Port Elizabeth, South Africa). While the use of asepsis and antibiotics in fish surgery has become controversial (see Mulcahy, 2011; Jepsen et al., 2013), infections are a risk in fish surgery especially when the fish is released back into a potentially contaminated environment (Jepsen et al., 2013). Water quality issues are present in the Thukela catchment, including high nutrient and faecal microbe concentrations (DWS, 2017), and the state of the Zingela stretch is unknown.

Accordingly, all tagged eels were administered, intramuscularly, with Terramycin® (Zoetis, Sandton, South Africa) containing oxytetracycline (1 ml/kg) to lower the risk of post-surgery infection. Additionally, wound gel care (Aqua Vet, Lyndenburg, South Africa) was applied to the incision site to reduce potential inflammation as per the South African Inland Fish Tracking Programme (FISTRAC) (O'Brien *et al*., 2014). In the last stage of the tagging procedure, the continuous flow of anaesthetic bath was changed for clean fresh river water, allowing for a quicker post-surgery recovery. Eels were then placed in a holding bucket with fresh oxygenated river water. The tagging procedure lasted 3–5 min and recovery from anaesthesia took 5–15 min. Eels were monitored for a minimum of 30 min after recovery before being released back to the river at the capture site.

Eels were manually tracked from the riverbank and from a kayak between October 2018 and August 2019. Tracking occurred daily from October to January 2019, and then daily for 10–15 consecutive days per month from February 2019 onwards. To assess the survival and health of the tagged individuals, fyke nets (n = 12) were set for 5– 6 consecutive nights monthly between February and July 2019. Recaptured eels were anaesthetised (method as above), identified by tag frequency, measured, weighed and photographed, especially in the incision region.

Between October 2018 and January 2019, 38 eels (*A. bengalensis* n = 15, *A. marmorata* n = 12, *A. mossambica* n = 11) were captured within the Zingela river stretch. Their size ranged from 215 to 1450 mm and their weight from 120 to 7900 g. Nineteen eels, comprising three species, were tagged (Table 1): African mottled eel *A. bengalensis* (n = 9), giant mottled eel *A. marmorata* (n = 8) and longfin eel *A. mossambica* (n = 2) (Table 1).

A total of 1753 locations were collected for the tagged eels from October 2018 to August 2019. The number of locations recorded per individual ranged from 18 to 152, corresponding respectively to 52 and 304 days after tagging. One individual (9) quickly left the study area, before all eels were tagged on the 8 January 2019. At the end of our study in August 2019, nine individuals (47% of all tagged eels) had tags that were still transmitting. Based upon the assumption that tag movements > 20 m (~ 4 times measured tag location error) between consecutive locations reflect a live tagged eel (Supplementary Table S1), 17/19 (89.5%) tagged eels survived 2 months or greater and, 9/13 (69.2%) eels tagged between October and December 2018 survived for at least 8 months. During the course of the study, only one individual (9) was confirmed outside the study area in January 2019. Every time a tag went missing, we searched the entire stretch of accessible river. It was the case when individual 10 stopped transmitting in March and individual 16 in May: none of these tagged eels were found in the study area or direct vicinity, it is assumed these individuals either left Zingela or that the battery failed. In June, six individuals were lost (3, 7, 8, 11, 12, 19): within the same week. However, no apparent adverse events (no change in flow, predators or fishing pressure) were observed, the end of the battery life was assumed as little long distance movements were observed beforehand.

Seven eels were recaptured (Table 1), with all displaying an advanced or complete state of healing (Fig. 1). However, slight inflammation at the incision and/or antenna exit sites was noted in some eels, and stitches were present up to 91 days after tagging. Whip antennas were mostly in good condition with little or no oxidation evident (Fig. 1-A2 and C2), but one broken antenna (about 2 cm away from the attachment point to the tag) was observed (Fig. 1-B2), with no significant change in signal strength.

For two individuals, tag expulsion was suspected but with no obvious expulsion site apparent, and complete healing of both the insertion site and antenna exit point (individuals 4 and 15). Both individuals showed obvious scarring marks suggesting the presence of stitches at an earlier stage. Scanning with a receiver confirmed tag expulsion for individual 4 and it was retagged as tags were still available at that stage of the study. After being tagged again, this eel's replacement tag was still transmitting at the end of the study and showed movement consistent with normal eel activity. The original expelled tag was stationary but was not recovered due to depth and high turbidity. When individual 15 was recaptured the presence of a potential tag was, unfortunately, not checked with a receiver; it is therefore uncertain if the tag was expelled (Fig. 1-D2). This tag was static and still transmitting at the end of the study in August 2018 from a shallow and rocky area, but attempts to retrieve the tag were not successful.

Four recaptured eels exhibited an increase in body mass suggesting that feeding and growth resumed after tagging (Table 1). Two individuals lost substantial body mass (9.7% and 16.0%, Table 1) and could suggest a tagging effect. However, these changes need to be viewed with care as captured eels were often observed feeding on top minnows (*Enteromius* spp.) and yellow fishes (*Labeobarbus* spp.) within the fyke nets, potentially affecting mass on capture, recapture or both. In terms of length, no substantial changes were observed.

While impacts of telemetry tagging are well documented for many fish species in temperate areas this is a largely undocumented topic in Africa. Less than 40 papers are available for African inland fish telemetry studies. Most attempts for recapture were unsuccessful (Baras *et al*., 2002; O'Brien *et al*., 2012). Mlewa *et al*. (2005) were the only researchers to observe live recapture of fishes (Lungfish, *Protopterus* spp.) and complete healing with no infection at the incision site and achieved a recapture rate of 8%. Other tags were also recovered after predation by birds (Thorstad *et al*., 2004) and capture in fisheries (Økland *et al*., 2005), but the effects of tagging were not documented. In comparison, our recapture rate was found to be relatively high, 37.5 and 44% for *A. marmorata* and *A. bengalensis* respectively. This can be explained by our high effort in obtaining recaptures as well as the typical resident behaviour shown by the tagged eels.

While tag expulsion can be a problem when studying fish behaviour (Økland *et al*., 2003; Mlewa *et al*., 2005), there are many advantages to using internal tags in movement studies of eels. Internal tags have been reported having higher retention rates than external tags for silver American eel *A. rostrata* (Lesueur 1817) (Cottrill *et al*., 2006). Few studies have used radio- or acoustic-telemetry to investigate eel behaviour during their inland yellow-stage: *A. anguilla* (Linnaeus 1758) have been successfully tracked with surgically implanted whip antenna radio tags with no observed expulsion (Baras *et al*., 1998; Ovidio *et al*., 2013) as have American eels *A. rostrata* (Lamothe *et al*., 2000; Thibault *et al*., 2007). In New Zealand, Jellyman and Sykes (2003) observed a tag loss rate of surgically implanted tags of 25% for the shortfin eel *A. australis* and 23% for the longfin eel *A. dieffenbachii* Gray 1842. Low expulsion rates (5%) have also been observed for *A. australis* with injected passive integrated transponder tags (Jellyman and Crow, 2016). In our present study, with two cases of tag expulsion, we reached 12.5 % of tag loss for *A. marmorata* and 11% for *A. bengalensis*, while no tag loss was suspected for *A. mossambica*.

Considering the advanced state of healing for all recaptured eels, with no infection and little inflammation visible, and the low rate of confirmed expulsion, internal tagging for these three species of eel appears to be a viable option to study the movements of eels in South African rivers. Attention to the choice of study site should, however, be applied as the present study area is considered to have relatively good water quality as well as low anthropogenic user pressure, thus potentially lowering the risk of post-surgery infection or mortality.

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Figure 1 - Photographs of recaptured eels showing the state of healing. Individual 3 (*A. marmorata*) full body (A1) and zoom on wound area (A2, anterior to right) 28 days after tagging; individual 18 (*A. bengalensis*) full body (B1) and zoom on wound area (B2, anterior to left) showing the broken antenna 78 days after tagging; individual 19 (*A. bengalensis*) full body (C1) and zoom on wound area (C2, anterior to right) 78 days after tagging; individual 15 full body (D1) and zoom on wound area (D2, anterior to right) 112 days after tagging, showing complete healing after a potential expulsion.

Table 1 Details of all eels (*Anguilla* spp.) radio tagged in the Thukela River, South Africa, including body length, body mass and time elapsed for recaptured radio-tagged eels.

ID	Species	Date of capture	Body mass at capture (g)	Body length at capture(mm)	Recapture			Total time tracked (days)	Final fate of tagged fish
					Difference in body length	Difference in body mass	Days elapsed		
1	*A. mossambica*	23/10/2018	855	650				>304	Still transmitting 23/08/2019
2	*A. mossambica*	23/01/2019	480	570				>215	Still transmitting 23/08/2019
3	*A. marmorata*	25/10/2018	4700	1300	+10 mm +0.8%	n/a	28	239	Last detection on 21/06/2019
4	*A. marmorata*	25/10/2018	7800	1380	-30 mm -2.2%	+100g +1.3%	93	>302	Tag expelled in Dec 2018, retagged and still transmitting 23/08/2019
5	*A. marmorata*	28/10/2018	4200	1180				>299	Still transmitting 23/08/2019
6	*A. marmorata*	20/11/2018	955	770				>276	Still transmitting 23/08/2019
7	*A. marmorata*	21/11/2018	5100	1270				213	Last detection on 22/06/2019
8	*A. marmorata*	22/11/2018	2080	1010	0	+150g +7.2%	66	210	Last detection on 20/06/2019
9	*A. marmorata*	05/12/2018	765	700				14	Located out of study area
10	*A. marmorata*	24/01/2019	6970	1450				58	Last detection on 22/03/2019
11	*A. bengalensis*	27/10/2018	4550	1250	0	-730g -16%	91	232	Last detection on 16/06/2019
12	*A. bengalensis*	20/11/2018	4045	1190				214	Last detection on 22/06/2019
13	*A. bengalensis*	22/11/2018	820	770				>274	Still transmitting 23/08/2019
14	*A. bengalensis*	22/11/2018	1630	955				>274	Still transmitting 23/08/2019
15	*A. bengalensis*	23/11/2018	1650	910	+5mm +0.6%	+160g +9.6%	112	>273	Still transmitting 23/08/2019
16	*A. bengalensis*	04/12/2018	1485	850				170	Last detection on 23/05/2019
17	*A. bengalensis*	24/01/2019	3040	1090				>211	Still transmitting 23/08/2019
18	*A. bengalensis*	27/01/2019	3435	1210	0	+300g +8.7%	78	203	Last detection on 18/08/2019
19	*A. bengalensis*	27/01/2019	5680	1260	0	-550g -9.7%	78	146	Last detection on 22/06/2019

Supplementary Table S1. Monthly numbers of movements exceeding 20 m between consecutive radio locations per tagged eel (*Anguilla spp.*) in the Thukela River. No tracking was carried out in July 2019.

	Number of movements > 20 m per month per individual																			
	id 1	**id 2**	**id 3**	**id 4**	**id 5**	**id 6**	**id 7**	**id 8**	**id 9**	**id 10**	**id 11**	**id 12**	**id 13**	**id 14**	**id 15**	**id 16**	**id 17**	**id 18**	**id 19**	**Monthly Mean**
October	6		3	7								7								5.8
November	19		11	14	10	3	4	2			9	14	2	2	3					7.8
December	10		17	7	0	4	19	13	1		8	7	11	10	15	8				9.3
January	3	2	13	1	2	2	14	14	3		16	1	9	4	9	9				6.8
February	4	4	5	5	0	5	5	1		9	0	5	3	0	3	3	8	2	3	3.6
March	1	3	2	3	3	2	2	1		4	7	3	1	2	5	5	6	4	3	3.2
April	4	2	8	8	4	10	8	0			10	8	6	2		9	6	5	6	6.0
May	6	2	5	1	3	3	6	0			8	1	2	0		1	2	7	7	3.4
June	2	1	4	1	3	0	4	0			5	1	1	0			6	1	1	2.0
August	3	0		0	4	4						0	0	0			6	0		1.7